Fabrication of Gold Arrays for Electrochemical Detection of Cancer Biomarkers

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Introduction

- Cancer statistics
  - Cancer is the 2nd leading cause of death
  - Approx. 596,000 patients per year
- Early Detection of cancer
  - Improve prognosis for future patients
- Cancer biomarker
  - Any measurable or observable factors in human body that indicates cancer or related diseases
  - Proteins, mutated DNAs, cell deaths, and physical symptoms
  - **Interleukin 6 (IL-6)**
- Enzyme Linked Immunosorbent Assay (ELISA/Immunoassay)
  - Use to detect and quantify proteins based on antibody-antigen interaction and specificity
  - 98/384 wells

1. Rusling, *Analyst* 2010 (135) 2496-2511
2. U.S. Center of Disease Control and Prevention

Goal

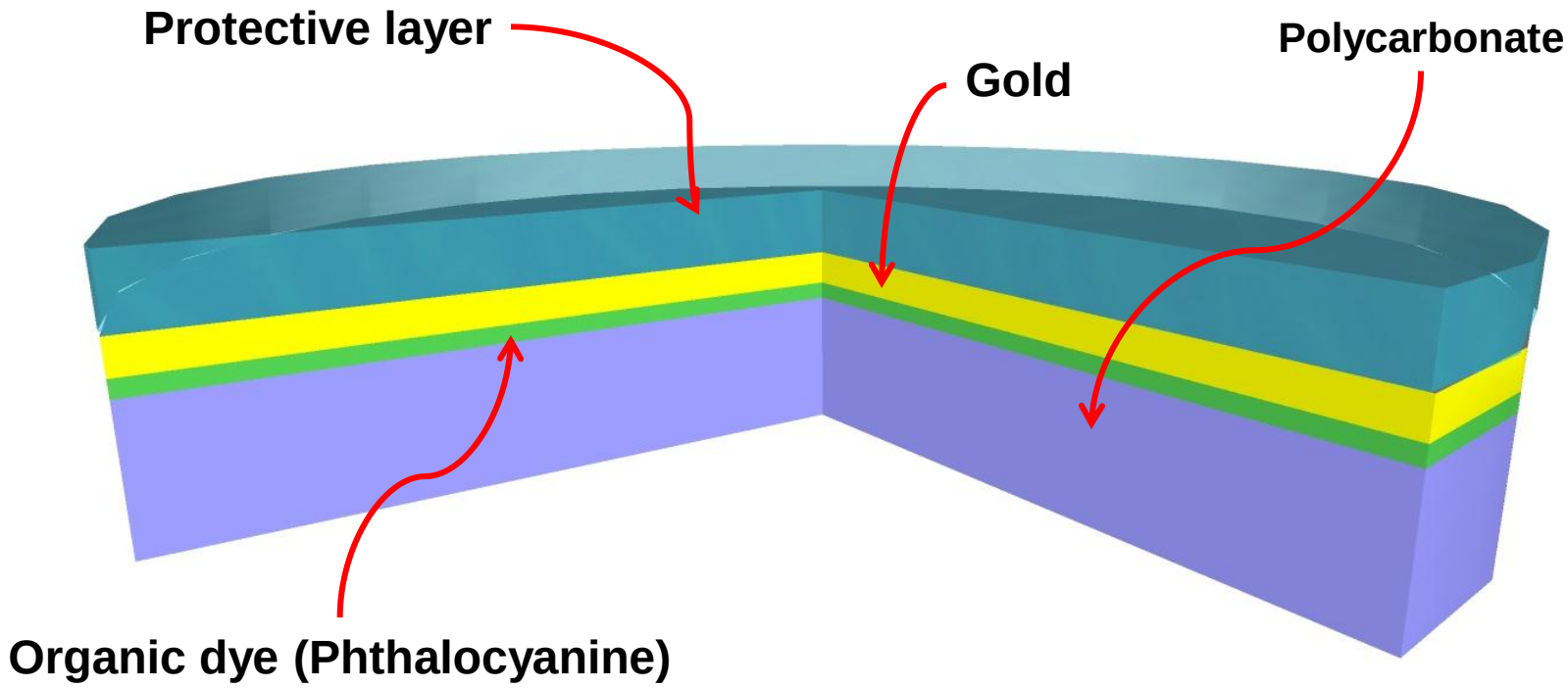
- Inexpensive and easy fabrication method for electrochemical arrays
- Integration with Microfluidics
- Point of Care device

Compact Disc

Arrays

Gold CD-Rs

- 650 MB Compact Disc Recordable
- Relatively Cheap – $1.5 per disk
- Easy to prepare
  - Can be cut into desired shape for different applications
- 50 to 100 nm single layer of sputtered gold (99.9% pure)



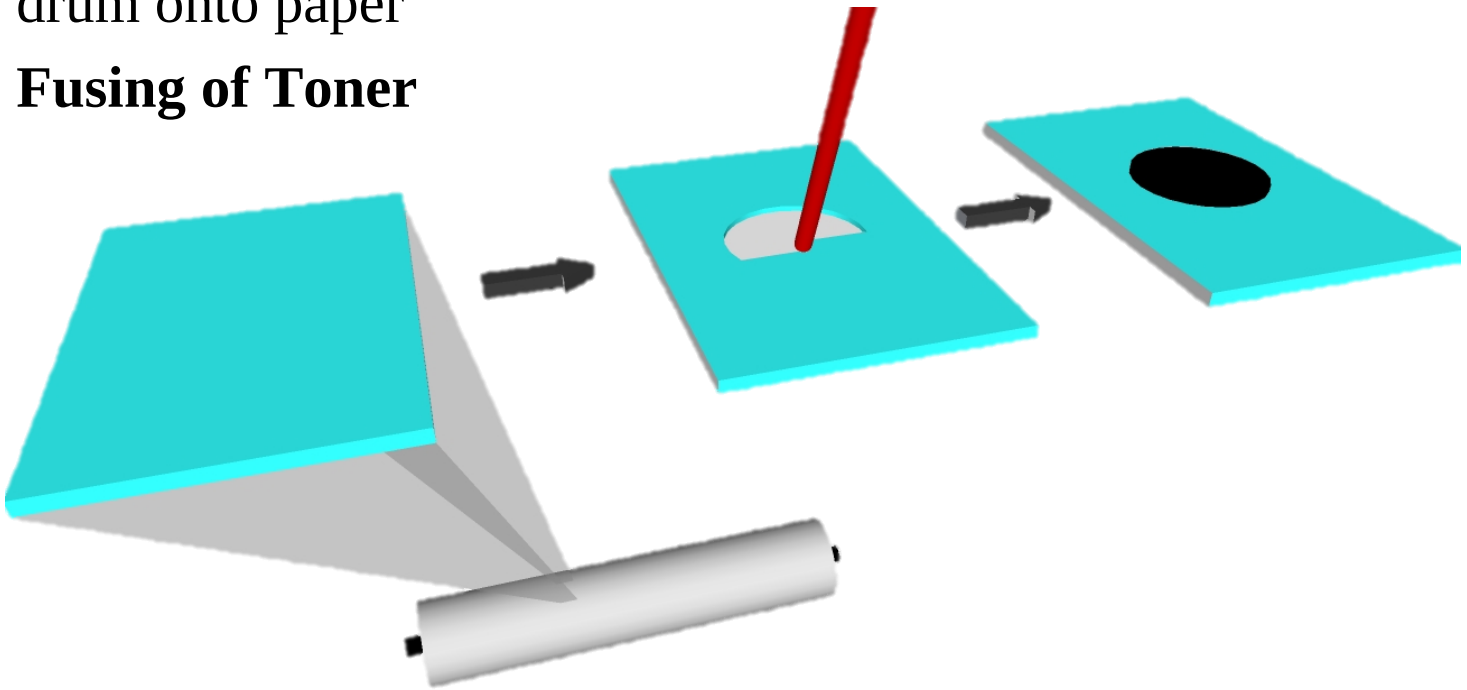
LaserJet Printing

- Reproducibility of printed patterns
- LaserJet Printer
  - HP LaserJet 1020 (600 dpi)
  - Styrene acrylic copolymer (melt at 125 ℃)
  - Iron Oxide

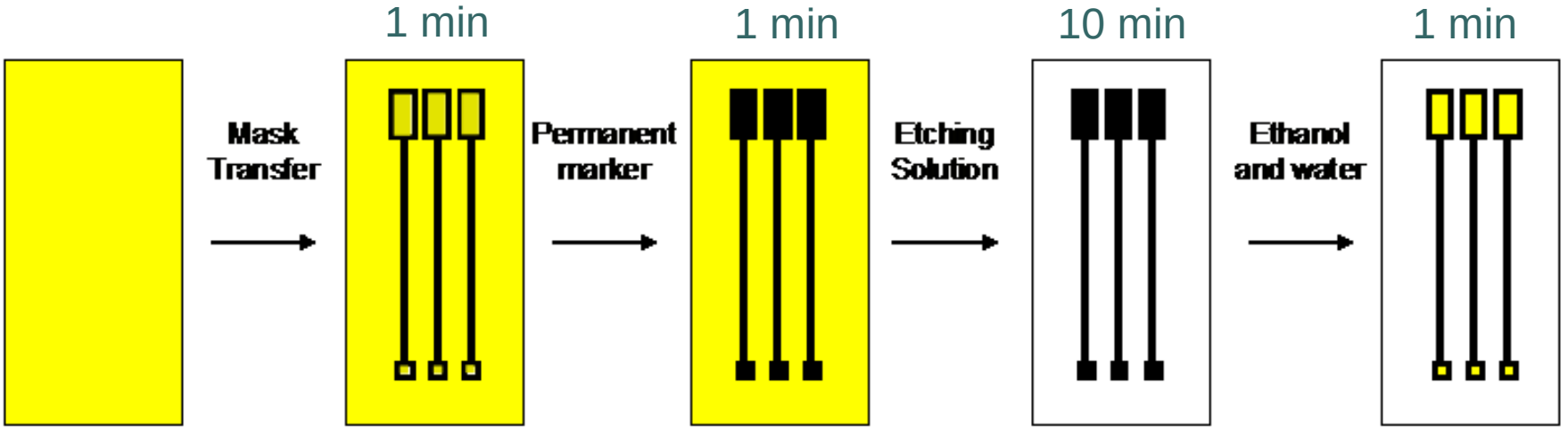
Cost: <$200

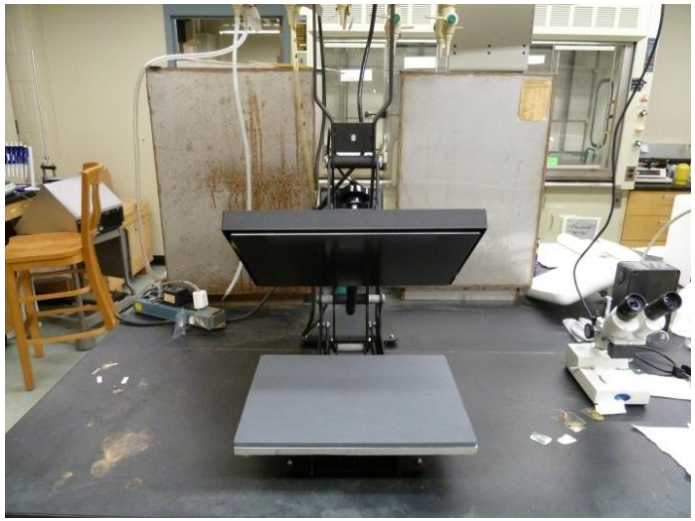
Printing process

- **Charging the photoconductor drum**: Photoconductor surface is charged
- **Exposure to light**: The charged surface is exposed to a laser
- **Development**: Negatively charged toner particle is brought to the photoconductor drum
- **Image transfer**: Toner is transferred from the photoconductor drum onto paper
- **Fusing of Toner**



Gold Arrays form CD-R

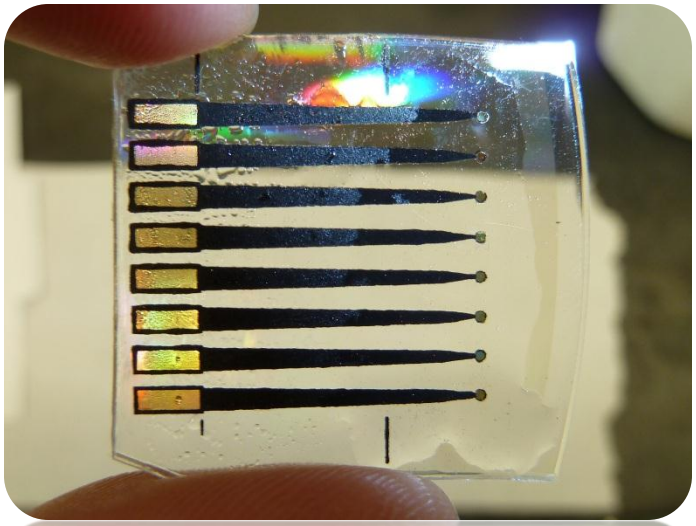




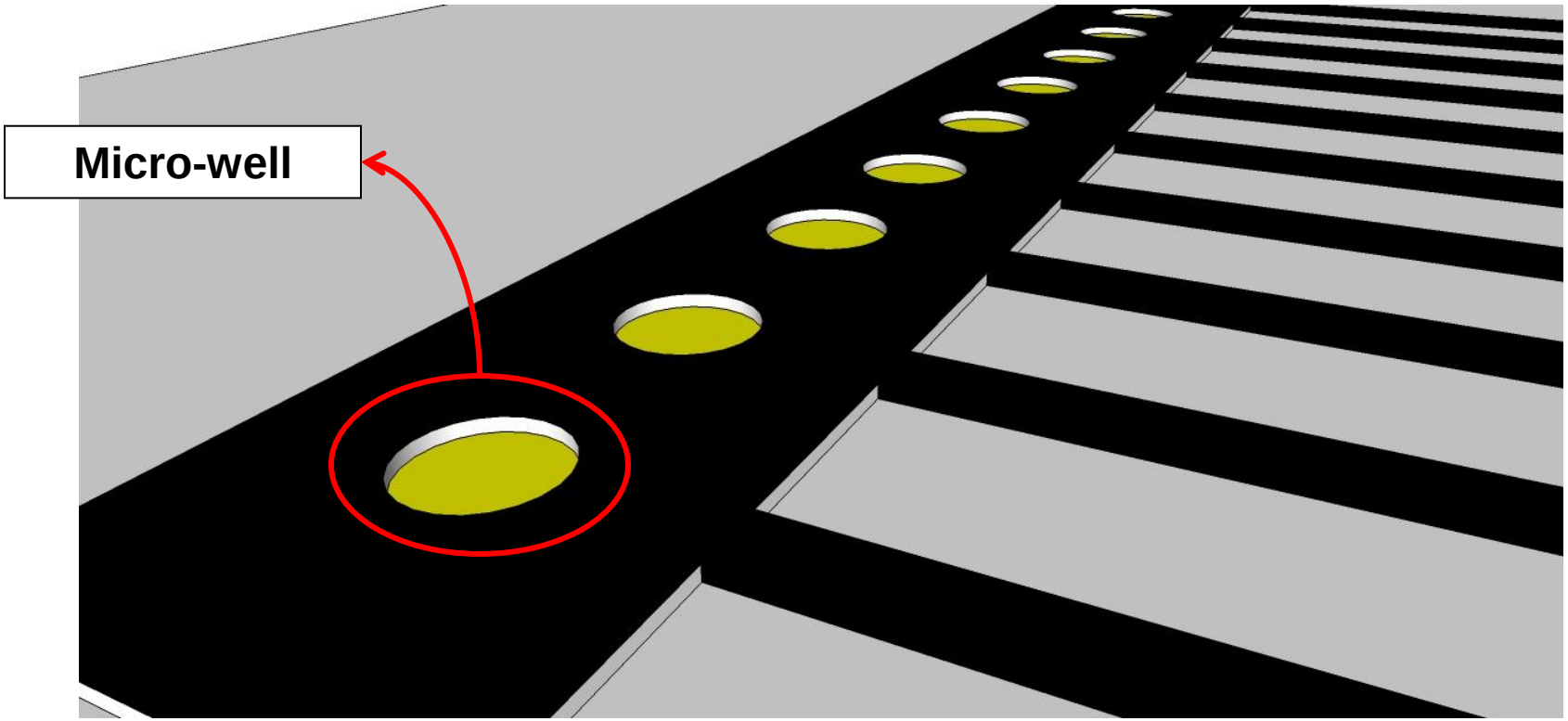
3. Deniel, et. al. *Electrochem Comm* 2003

Electrochemical arrays

- Electrode area reproducibility: ±10 % (n=8)
- Cost: $0.18 per array
- Time: Approx. 1hr for ~8 arrays
- Resistance: 21. 4 ±0.5 Ω



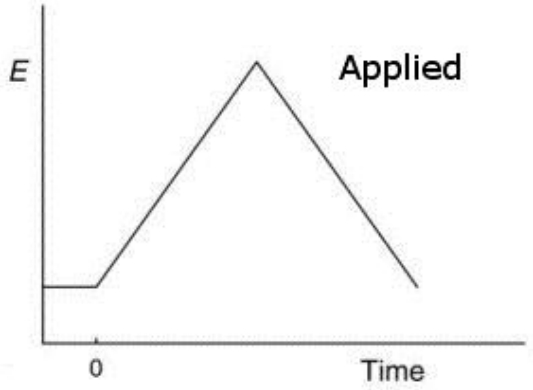
Micro-wells

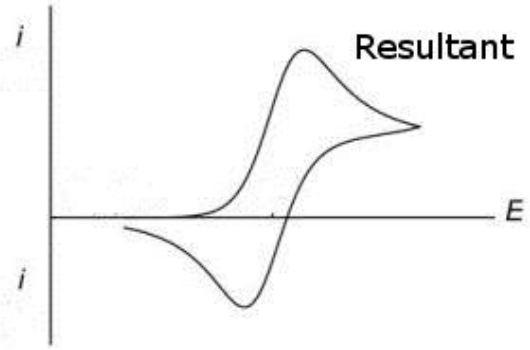


Micro-wells (cont.)

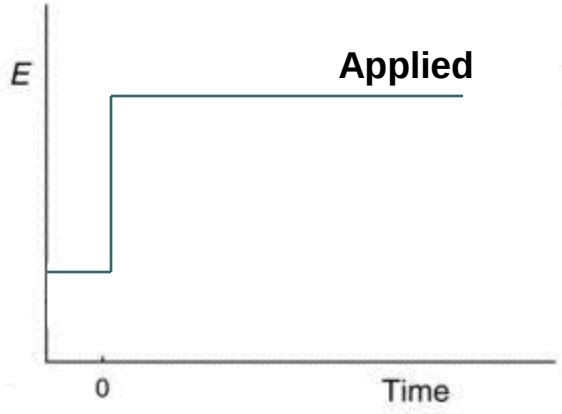
Electrochemical methods

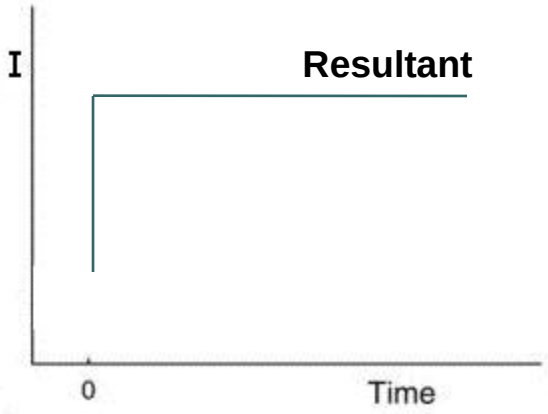
- Electrochemical cell
  - 3 electrodes system
- Cyclic voltammetry





- Amperometry

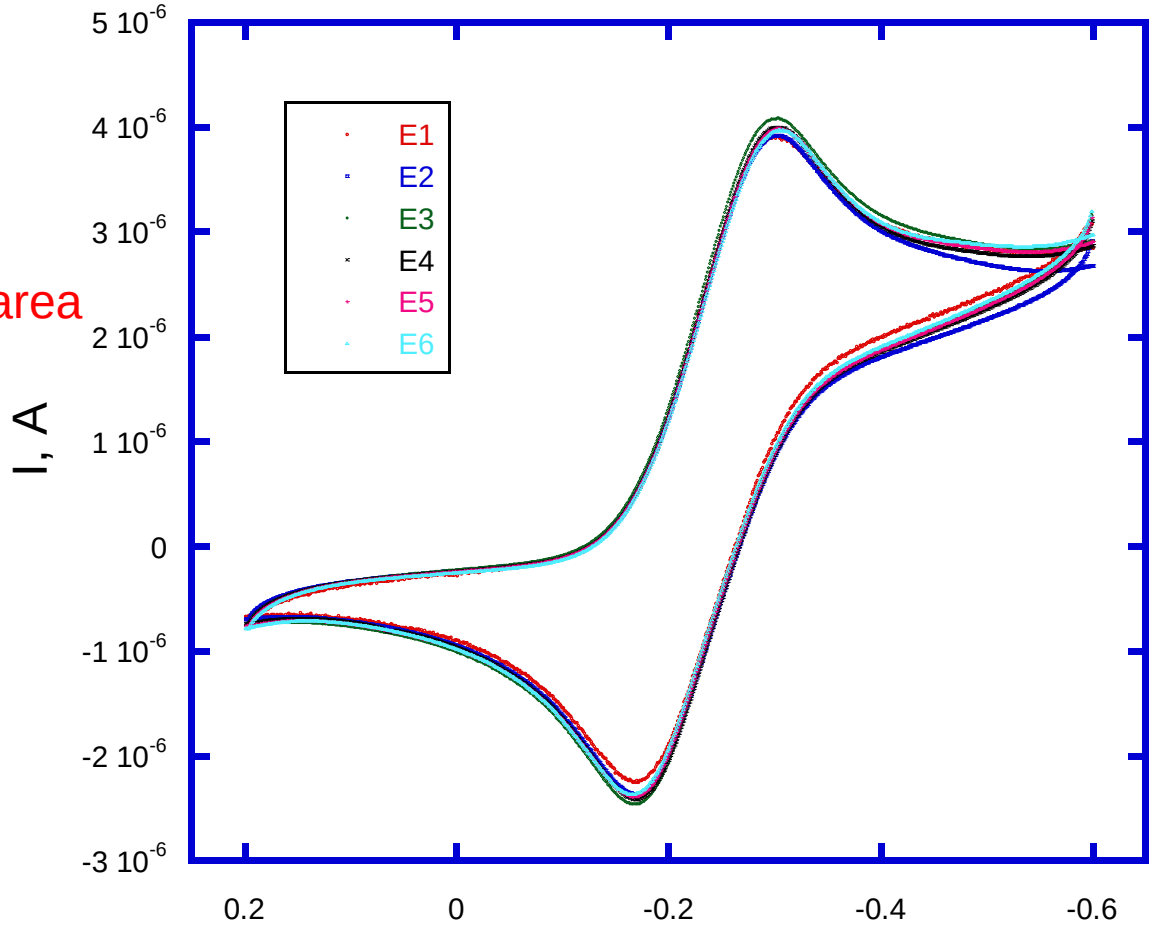




Surface Area

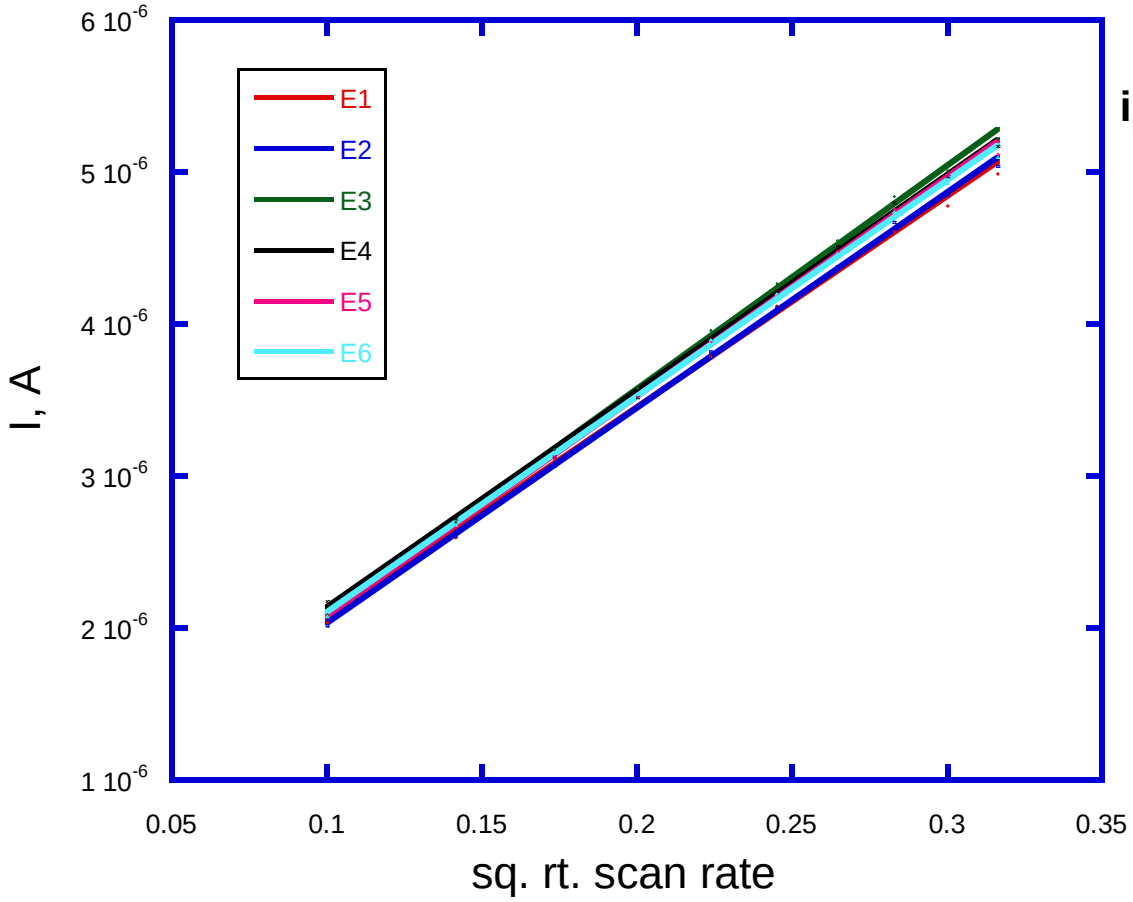
$$i_p = (2.687 \times 10^5)\, n^{3/2}\, v^{1/2}\, D^{1/2}\, A\, C$$

i_p – peak current
n – number of electrons
v – scan rate
D – diffusion coefficient
A – electro-active surface area
C – concentration of probe



CV at 50 mV s^{-1} of gold CD-R array in 5 mM $Ru(NH_3)_6Cl_6$ and 0.1 M Sodium triflouroacetate (NaTFA)

Surface Area (cont.)



Randles Sevcik Equation

$$i_p = (2.687 \times 10^5)\, n^{3/2}\, v^{1/2}\, D^{1/2}\, A\, C$$

Surface Area:

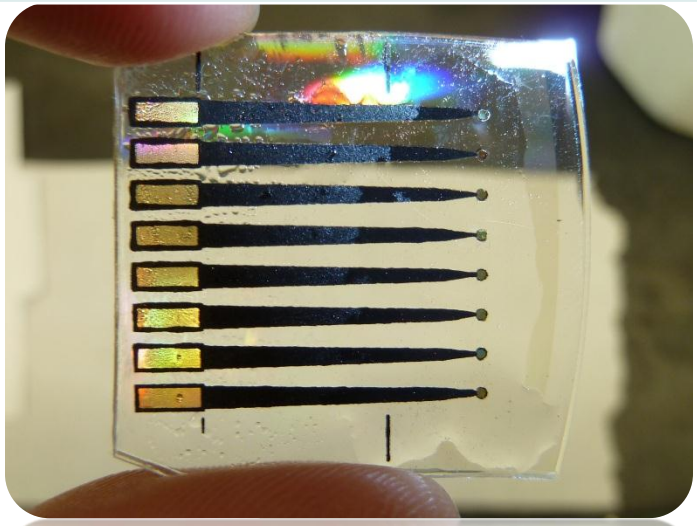
0.00427 ± 0.00007 cm^2

~1.6% RSD

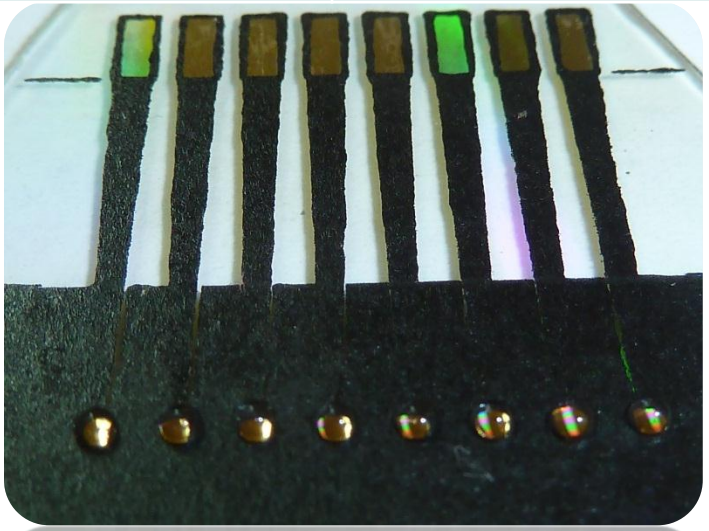
75% of geometric area

Summary

	Old design	New design
Surface Area (cm^2)	0.036	0.042
Reproducibility	~10%	~2%
Hold < 1 µL drop of reagents	No	Yes



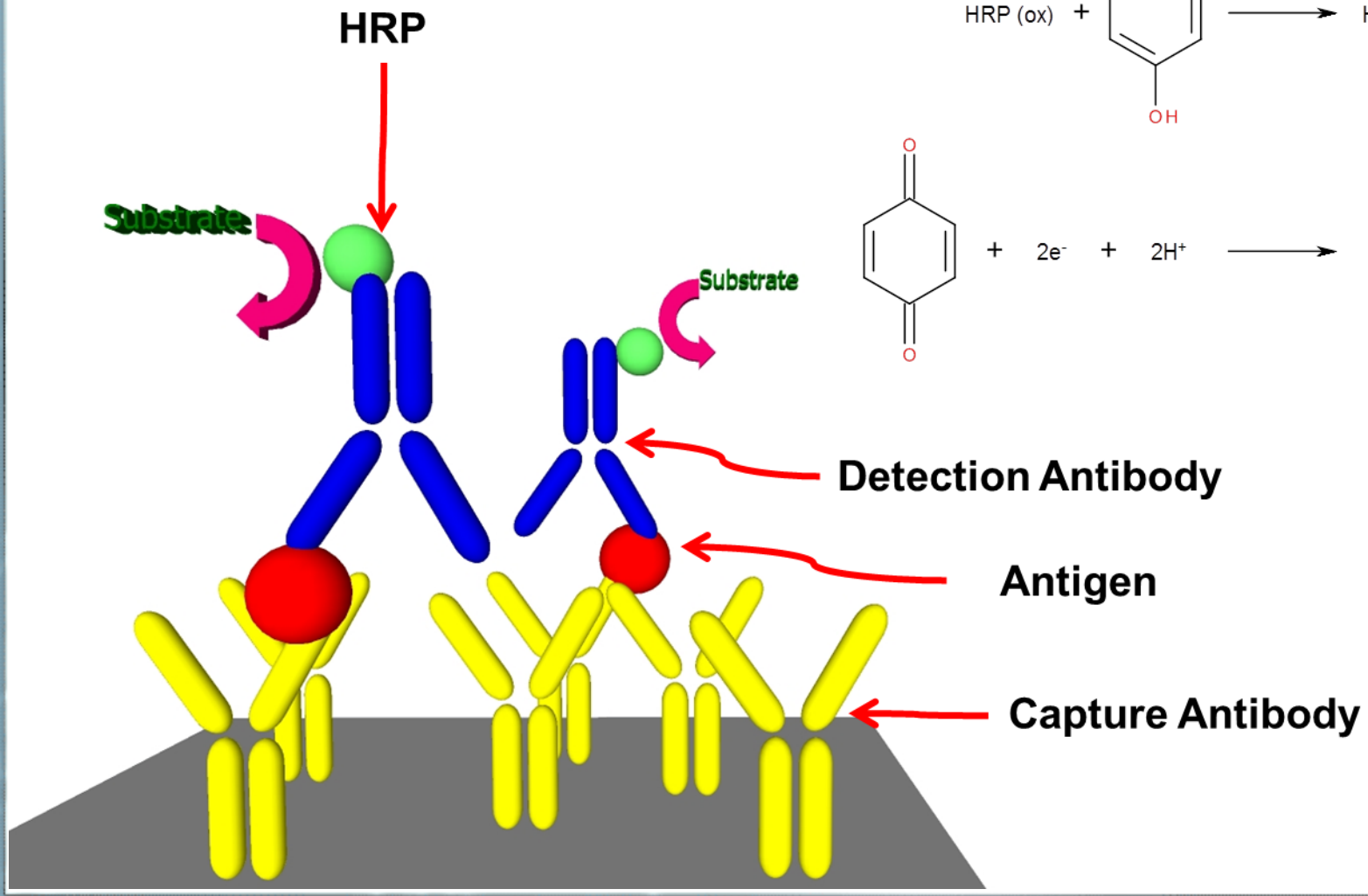
Old

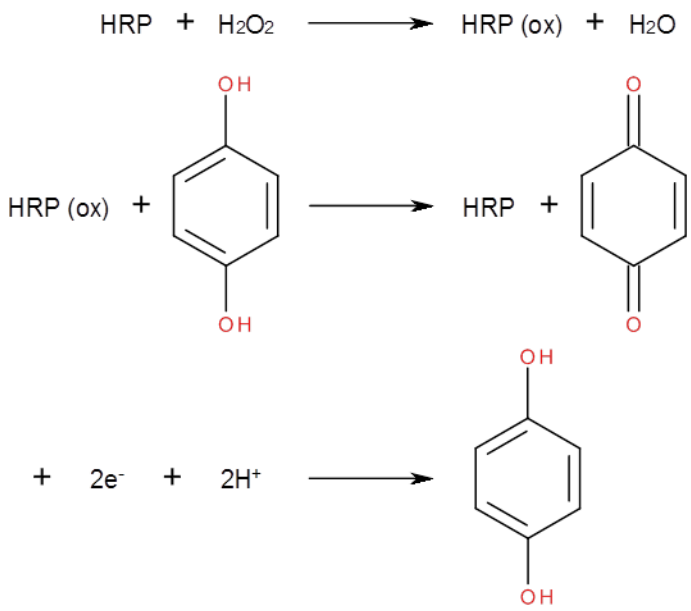


New

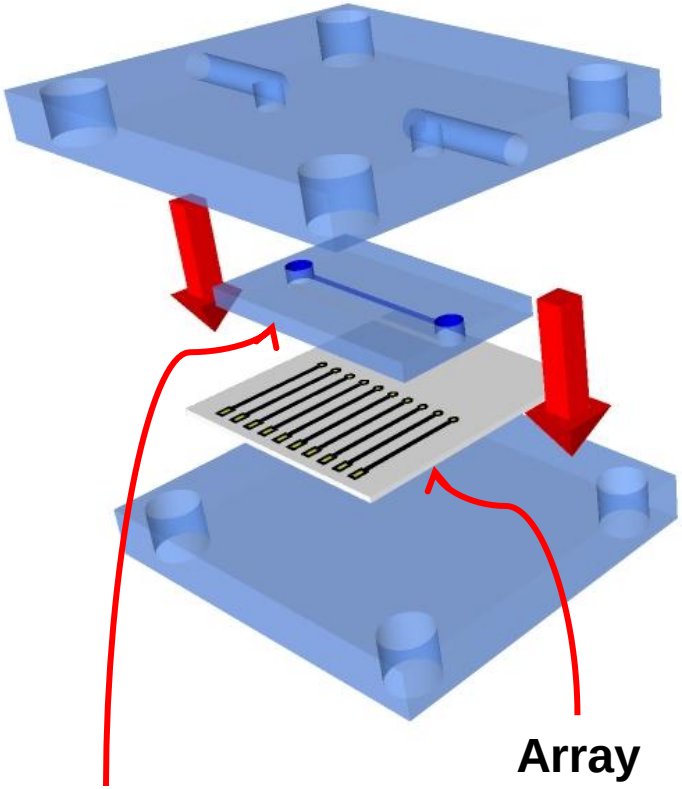
Sandwich Immunoassay

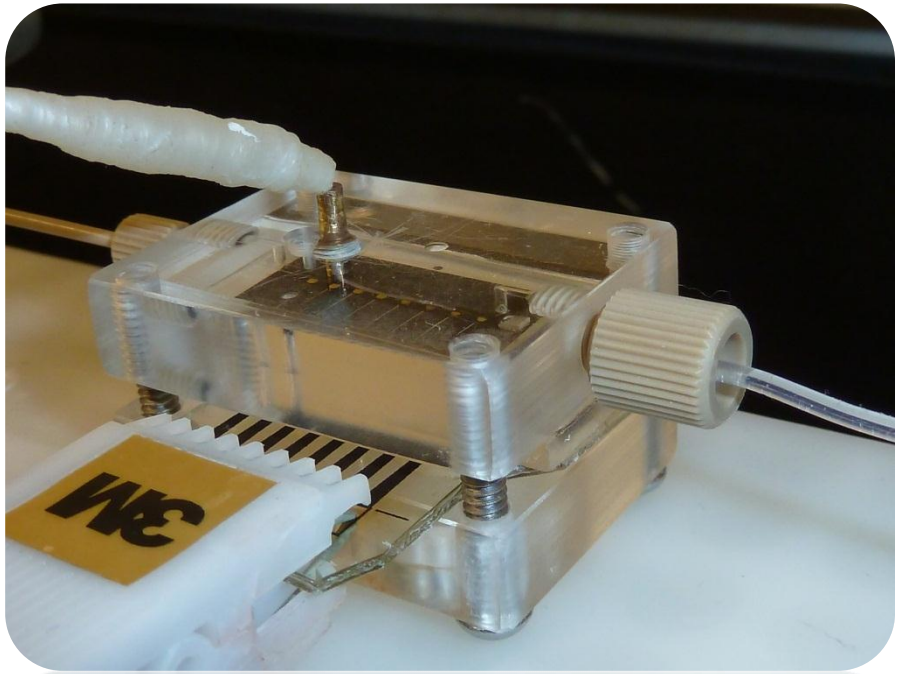
Interleukin-6 (IL-6)
Normal patient range: < 6 pg/mL
Cancer patient range: > 20 pg/mL



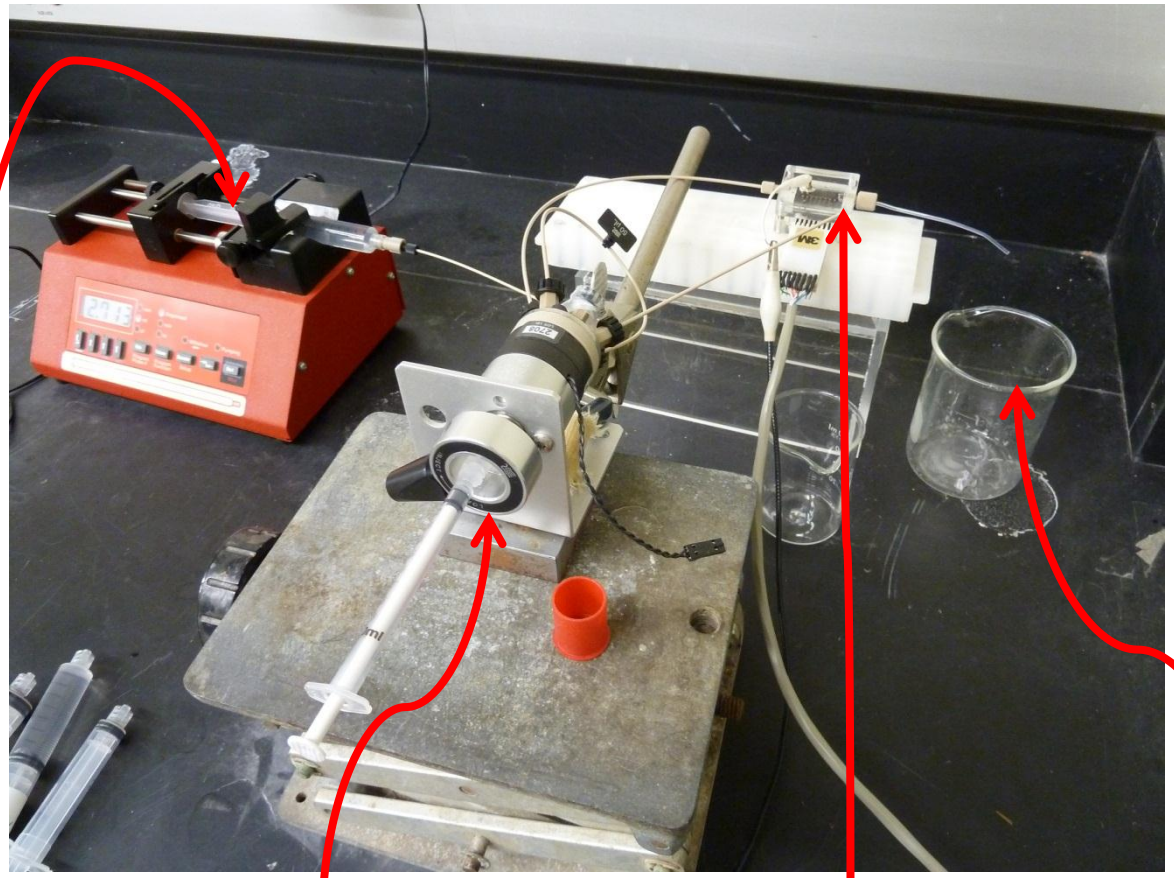


Integration with Microfluidic Device





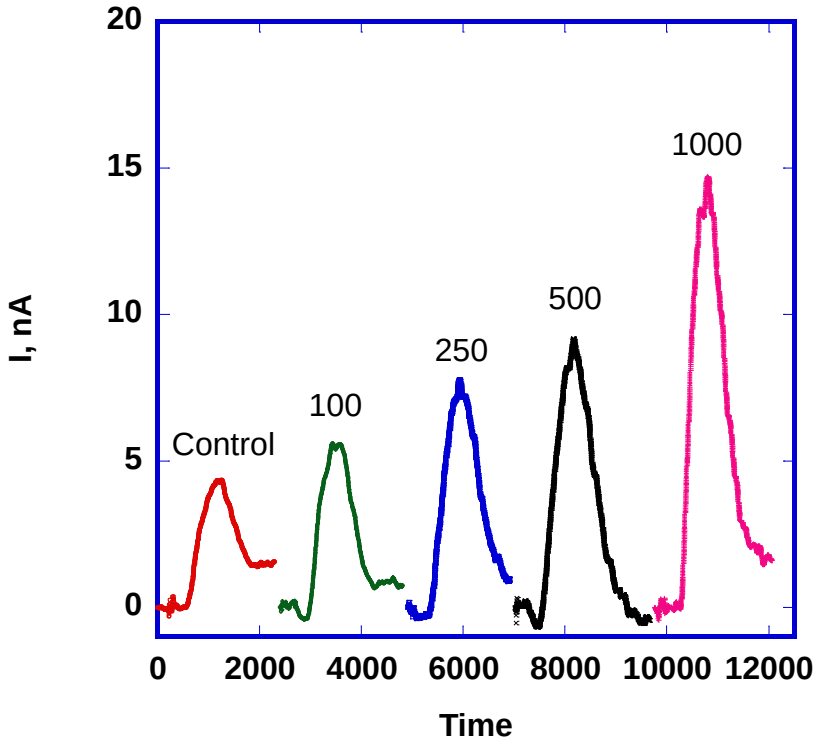
Instrument Setup

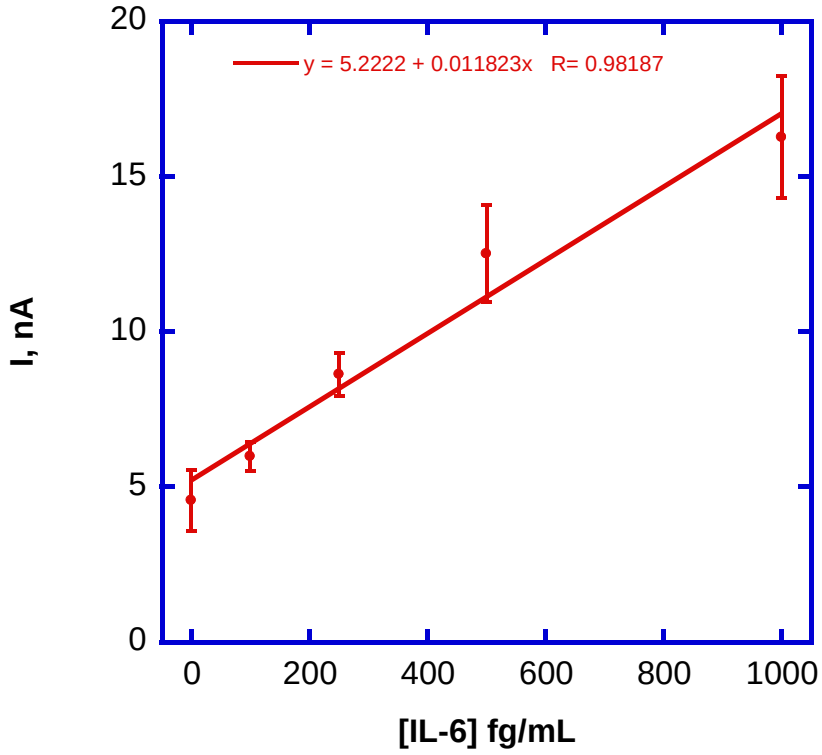


Pump → Injector Valve → Microfluidic device → Waste

Microfluidic device → Potentiostat

Calibration Plot for Interleukin-6





Amperometric response of Au/MPA/Ab_1/Ag/Ab_2/Strep-HRP with 1 mM HQ and injection of 100 uM H_2O_2 at -0.3 V vs. SCE in microfluidic device

Limit of Detection:
100 fg/mL

Conclusion

- Fabricate gold electrode array at low cost (~$0.20)
- Reproducible electrode areas (~2% RSD)
- Successful development of immunosensor for IL-6
  - Low detection limit: 100 fg/mL
- Integration with microfluidics
- Multiplexing
- Point of Care device

Acknowledgment

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- Group members
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Thank You!

Questions?